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Contractile Effect of Ultraviolet Light on Isolated Thoracic Aortae of Rats

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ABSTRACT

Ultraviolet light radiation (UVR) did not affect resting tension of isolated thoracic aortae of rats. In aortic rings contracted with phenylephrine, however, UVR produced contractile and relaxant responses in preparations with and without endothelium, respectively. The contractile response was dependent upon the duration (10~320 sec) of irradiation, while the relaxation was not.

UVR-induced contractions in endothelium-intact rings were significantly potentiated by increasing the concentrations of phenylephrine from 10^{-7} M to 10^{-5} M, and also by addition of 10^{-6} M acetylcholine, 10^{-7} M isoproterenol and 3.5×10^{-8} M nitroglycerine. However, addition of 10^{-6} M phentolamine, or 10^{-7} M to 10^{-6} M LY83583 inhibited the contraction or reversed the contraction to a relaxation.

In endothelium-removed preparations the UVR-induced relaxation was attenuated by increasing concentractions of phenylephrine, and by addition of isoproterenol, nitroglycerin, phentolamine or LY83583.

These results suggest that UVR produces contractile and relaxant responses in rat thoracic aortae with and without endothelium, respectively, and that the contractile effect results from the inhibition of endothelium-derived relaxing factor (EDRF) release by UVR the inhibition of and/or is in part related to some endothelium-derived contractile factors (EDCFs).

Key Words: Ultraviolet light, thoracic aorta, contraction, cyclic GMP

INTRODUCTION

Furchgott *et al.* (1955) first observed that ultraviolet and visible lights relax thoracic aortae of rabbits precontracted with several vasoconstrictors. Since then, ultraviolet light radiation (UVR)-induced relaxations have been reported in a variety of vascular preparations, including rabbit (Furchgoot, 1971, Furchgott *et al.*, 1984) and rat thoracic aorta (Mikkelsen *et al.*, 1985; Lincoln *et al.*, 1985; Triggle and Bieger, 1990), and bovine mesenteric artery (Karlsson *et al.*, 1984; 1986). In a more recent study, contractions of rat aorta induced by KCl or norepinephrine (NE) were unaltered by UVR, while UVR elicited relaxant response in tissues contracted with a dihydropyridine calcium channel agonist, Bay K 8644 (Mikkelsen *et al.*, 1985), and treatment with (+) PN 202-791 or (−) Bay K 8644 of rat aorta precontracted with NE potentiated UVR-induced relaxation (Triggle and Bieger, 1990).

Although the detailed mechanism of UVR-induced relaxation is not completely understood,

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considerable evidence has been accumulated to suggest a similar mechanism to the nitrovasodilators. Nitrovasodilator- or UVR-induced relaxation is associated with increased levels of cyclic GMP (Furchgott *et al.*, 1984; Lincoln *et al.*, 1985; Karlsson *et al.*, 1984; 1986). In addition, the relaxation and elevation of cyclic GMP are inhibited by methylene blue and/or hemoglobin, inhibitors of guanylate cyclase activation (Furchgott *et al.*, 1984; Matsunaga & Furchgott, 1989; Lincoln *et al.*, 1985).

Recently, we found that UVR produces a contraction of endothelium-intact preparations but a relaxation of endothelium-removed ones in isolated aortae of rats. To our knowledge, this UVR-induced contraction has not been described previously and the present study was aimed at investigating the mechanism (s) of the contractile response to UVR using the isolated rat thoracic aorta with intact endothelium.

METHODS

Sprague-Dawley rats (200~300 g) of either sex were killed by dislocation of cervical cord. Thoracic aorta was dissected out and immersed in cold (4℃) physiological salt solution (PSS). The aorta was carefully cleaned of loose connective and adipose tissues with a special caution not to inflict damage to the endothelium, and was cut into rings (length 4~6 mm). Endothelium-removed preparations were made by gentle rubbing of the endothelial cell layers with a metal rod.

The aortic rings were then placed under 2 g-force resting tension in tissue baths conaining 37℃ PSS which was bubbled with a mixture of 95 % O_2 and 5% CO_2. Isometric tension of the rings was monitored by a force transducer (Grass FTO3) connected to a Grass polygraphy (7D). The rings were equilibrated for about 2 hours, which was followed by addition of 35 mM KCl and two subsequent washes. The removal of the endothelium was functionally checked by the addition of 10^{-6} M acetylcholine (ACh) to the rings precontracted with 10^{-6} M phenylephrine (PE). The composition (in mM) of PSS used was NaCl, 122; KCl, 4.7: $CaCl_2$ 2.0; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 15; glucose, 11.5; EDTA, 0.026; ascorbic acid, 0.12.

UV lamp (mineralight, UVP Inc.; wave length 365 nm; intensity 300 $\mu W/cm^2$ at 15 cm) was placed 2 cm from the tissue for 10 to 320 sec under dark condition using 5 W red lamp. The magnitude of changes induced by UVR was expressed as mean±SEM in terms of active tension (g) or mean±SEM of % changes obtained from the following equation: UVR-induced contraction (g) after an agent/UVR-induced contraction (g) before an agent×100. Statistical significance was examined using Student's paired or unpaired t-test.

Phenylephrine HCl, isoproterenol HCl, endothelin I and acetylcholine HCl were obtained from Sigma. Nitroglycerin was obtained from Marion, phentolamine methanesulfonate form Ciba-Geigy, and LY83583 (6-aniline-5, 8-quinolinedione) from Eli Lilly. LY83583 was dissolved in absolute ethanol to yield a 10^{-3} M stock solution which was serially diluted with distilled water, and other agents were dissolved in and diluted with distilled water.

RESULTS

Contractile and Relaxant Effects of UVR on Phenylephrine Inducted Tension

Neither the vascular ring with endothelium nor the ring without endothelium was affected by UVR (5 min) under resting tension. However, UVR elicited a rapid and reversible contraction of endothelium-intact rings contracted with 10^{-6} M PE, in which the magnitude of contraction was dependent upon duration (10 sec to 320 sec) of radiation (Fig. 1). Endothelium-removed rings contracted with the PE were rapidly and reversibly relaxed by UVR of which amplitude was gradually decreased inversely to the duration of radiation. The relaxation was maintained during the radiation but the original tension was immediately recovered following stopping the radiation (Fig. 1). The 10^{-6} M PE-induced tension (1.9±0.09 g, n=26) in endothelium-removed rings was significantly greater than that (0.8±0.12 g, n=72) in endothelium intact ones.

Endothelin, 10^{-9} and 3.5×10^{-9} M, produced constant contraction of endothelium-intact rings and

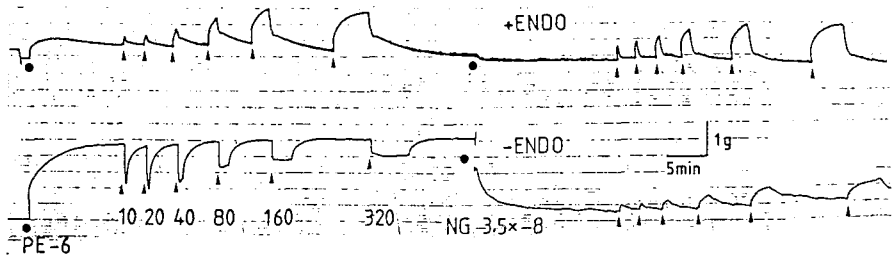

Fig. 1. Effects of nitroglycerin on contractile and relaxant responses to ultraviolet light radiation (UVR) iin an isolated rat thoracic aorta contracted with 10^{-6} M phenylephrine. Upper trace was obtained from an endothelial intact (+ENDO) ring and lower from an endothelium-removed (−ENDO) one. At dots the indicated drug was added to the bath. PE −6 and NG 3.5×−8 mean phenylephrine 10^{-6} M and nitroglycerin 3.5×10^{-8} M, respectively. Numerals indicate duration (sec) of UVR.

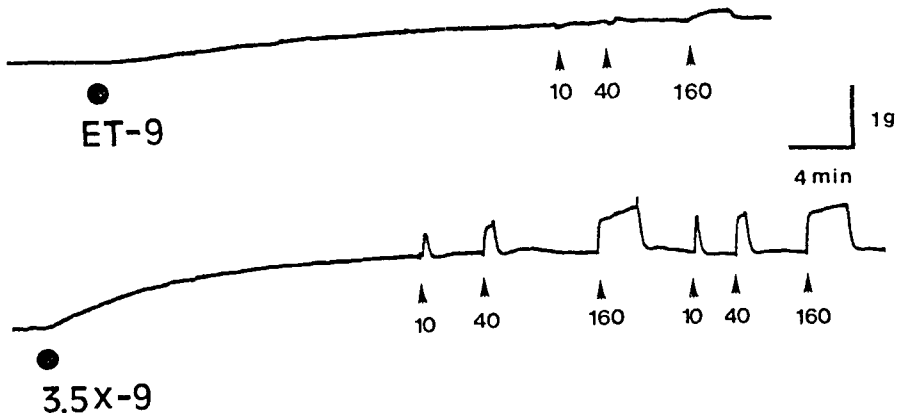

Fig. 2. A typical trace showing radiation time-dependent contractions of UV light to endothelin I (ET I)-induced contraction in an intact endothelial ring of a rat. Upper and lower trace were continously obtained in a ring. Numerals indicate radiation time (seconds). ET −9 and 3.5×−9 show 10^{-9} M and 3.5×10^{-9} M endothelin I, respectively.

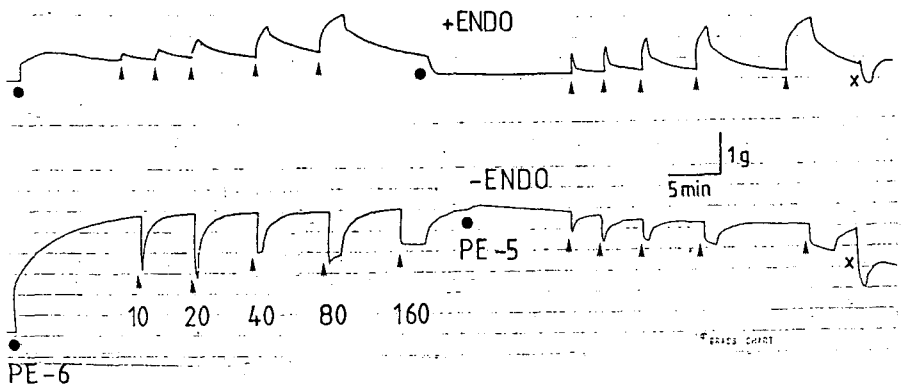

Fig. 3. Effects of cumulative dose 10^{-5}M of phenylephrine on contractile and relaxant responses to UVR in an isolated rat thoracic aorta contracted with 10^{-6} M phenylephrine. Legends are the same as in Fig. 1.

also UVR elicited radiation time-dependent contractions (Fig. 2).

Potentiation of UVR-Induced Contraction

Phenylephrine: Effects of 10, 40 and 160 sec UVR were observed at three serial concentrations (10^{-7}, 10^{-6} and 10^{-5} M) of PE in endothelium-intact rings. Mean tension induced by 10^{-7} M PE was 0.6 ±0.12 g(n=15) and UVR produced slight contractions. Then when 10^{-6} M PE produced a further contraction (0.8±0.02 g, n=22), UVR-induced contractions became significantly greater than those in 10^{-7} M PE-induced tension. While cumulative addition of 10^{-5} M PE relaxed the 10^{-6} M PE-induced tension itself, UVR-induced contractions were more greatly potentiated (Fig. 3 and 4). On the contrary, cumulative addition of 10^{-5} M PE in endothelium-removed rings slightly potentiated the 10^{-6} M PE-induced tension but UVR-induced relaxations were markedly inhibited (Fig. 3).

Acetylcholine: 10^{-6} M ACh relaxed 10^{-6} M PE-induced tension to the baseline in endothelium-intact rings, then UVR-induced contractions were significantly potentiated than those before addition of ACh (Fig. 5).

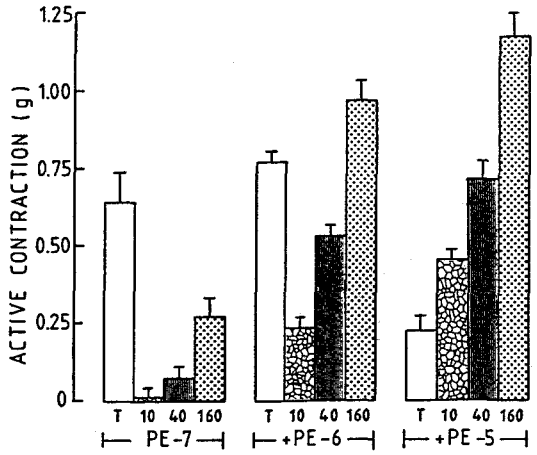

Fig. 4. Effects of cumulative doses of phenylephrine on contractile responses to UVR in endothelial intact rings of isolated rat thoracic aortae. All columns are mean±SEM from 13 rings. The indicated concentrations of phenylephrine (PE) were cumulatively added to the bath. Numerals show the duration (sec) of UVR and the columns indicate active contractions induced by the duration. Each T column shows active tension induced by the phenylephrine (PE) itself. Each PE -X shows log 10^{-X} M concentraction.

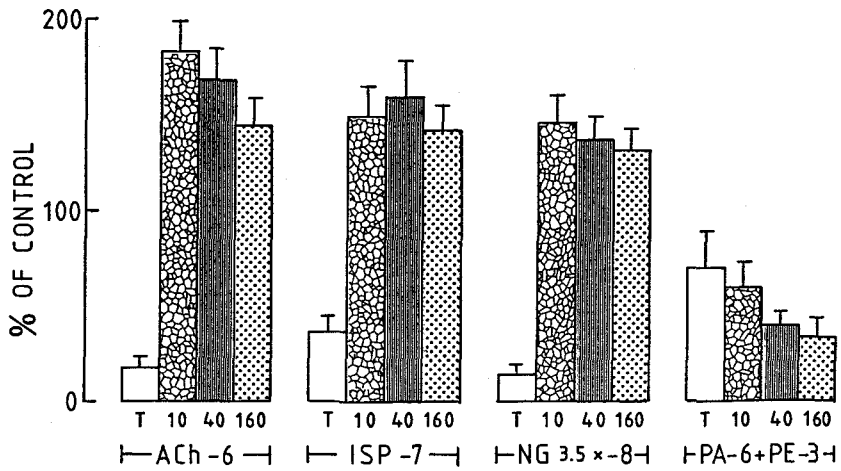

Fig. 5. Effects of various vasorelaxants on contractile reponses to UVR in endothelial intact rings of isolated rat thoracic aortae contracted with 10^{-6} M phenylephrine. % of control (Y axis) was obtained from % changes after the drug when the corresponding response before the indicated drug was taken as 100%. All columns are mean ±SEM from 15~21 rings. PA −6+PE −3 panel was obtained in rings contracted with 10^{-3} M phenylephrine in the presence of 10^{-6} M phentolamine. ACh: acetylcholine ISP: isoproterenol PA: phentolamine Other legends are the same as in Fig. 1 and 4.

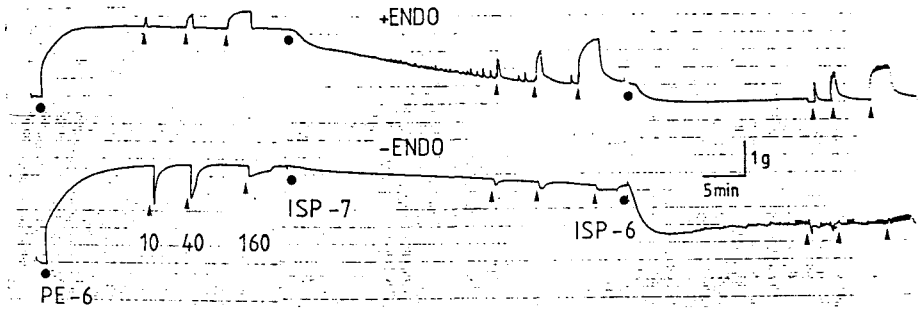

Fig. 6. Effects of isoproterenol on contractile and relaxant responses to UVR in an isolated rat thoracic aorta contracted with 10^{-6} M phenylephrine. ISP −7 and ISP −6 are isoproterenol 10^{-7} M and 10^{-6} M, respectively. Other legends are the sameas in Fig. 1

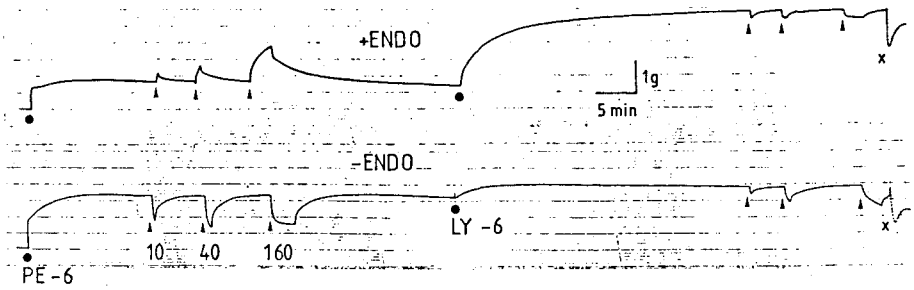

Fig. 7. Effects of LY83583 on contractile and relaxant responses to UVR in an isolated rat thoracic aorta contracted with 10^{-6} M phenylephrine. LY −6 is LY83583 10^{-6} M. Other legends are the same as in Fig. 1.

Isoproterenol (ISP): In endothelium-intact preparations, 10^{-7} M and 10^{-6} M of ISP markedly relaxed the PE-induced tension, then UVR-induced contractions were significantly potentiated than those before addition of ISP (Fig. 5 and 6). However, the same concentrations of ISP markedly relaxed the PE-induced tension in endothelium-removed rings, while UVR-induced relaxations were attenduated or abolished (Fig. 6).

Nitroglycerin (NG): 10^{-6} M PE-induced tension was markedly relaxed by addition of 3.5×10^{-8} M NG in both rings with and without endothelium. Then UVR-induced contractions in endothelium-intact rings were significantly potentiated than those before addition of NG, while UVR-induced relaxations in endothelium-removed preparations were reversed to slight contractile responses (Fig. 1 and 5).

Inhibition of UVR-Induced Contraction

Phentolamine (PA): 10^{-6} M PE-induced tension was completely abolished by addition of PA 10^{-6} M, and UVR-induced contractions were also abolished. Then, addition of higher concentration (10^{-3} M) of PE recontracted the tissue up to 75% of the tension-induced by 10^{-6} M PE and UVR-induced contractions were also in part recovered (Fig. 5).

LY83583: A new guanylate cyclase inhibitor, 10^{-7} and 10^{-6} M LY83583 (Schmidt *et al.*, 1985; MacLeod & Diamond, 1986) potentiated markedly 10^{-6} M PE-induced tension in endothelium-intact rings, but only slightly in endothelium-removed ones. Then UVR-induced contractile responses were reversed to relaxations in endothelium-intact rings and UVR-induced relaxations in endothelium-removed one was clearly inhibited (Fig. 7 and 8).

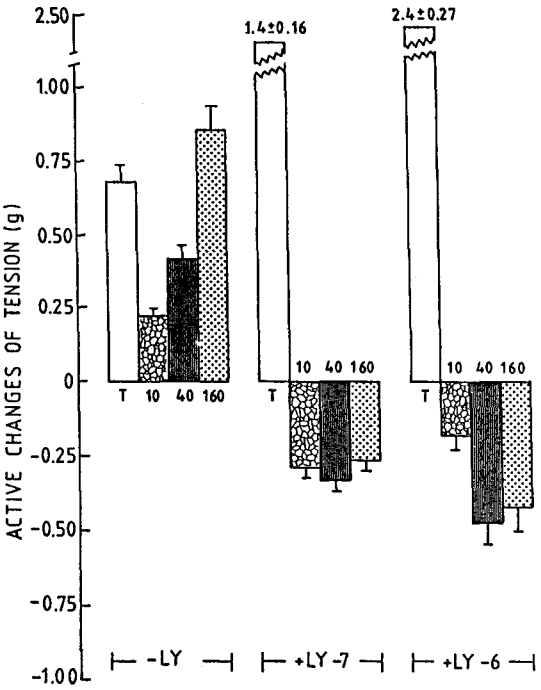

Fig. 8. Effects of LY83583 on contractile repsonses to UVR in endotheial intact rings contracted with 10^{-6} M phenylephrine. Left panel was obtained

DISCUSSION

The present study demonstrates that effects of UVR on the isolated thoracic aorta of rat was different according to the presense or absence of the endothelium. UVR produced time-dependent contractions in itact endothelial rings, while it caused relaxations in endothelium-removed ones. UVR-induced contraction has not been described thus far while UVR-induced relaxation has been reported by several groups. UVR-induced relaxation is known to be resulted from increases of cyclic GMP in vascular tissues through guanylate cyclase activation by UVR (Furchgott *et al.*, 1984; Lincoln *et al.*, 1985; Matsuhaga & Furchgott, 1989).

Furhermore, UVR-induced relaxations are not affected by the removal of the endothelium, being similar to the relaxant response to nitrovasodilators (Rapoport and Murad, 1983; Lincoln *et al.*, 1985). In the present study, however, UVR-induced relaxation appeared only in the endothelium-removed preparation contracted with PE, which was significantly inhibited by LY83583, a novel inhibitor of guanylate cyclase activation. This UVR-induced relaxation may also be mediated through increase of cyclin GMP synthesis.

The UVR-induced contractile repsonse was maintained during the radiation, and the original tension was immediately recovered after stopping the radiation. These results suggest that UVR releases an unknown contractile substance (s) in the vascular endothelium, and that the action duration of the substance is very short.

When PE-induced tension was completely abolished by addition of PA, UVR-induced contractions also disappeared. However, when the PE-induced tension was in part restored by addition of higher concentration of PE, the UVR contraction was also partly recovered. ACh, ISP and NG relaxed PE-induced tension to the baseline; nevertheless, UVR-induced contractions were significantly potentiated rather than inhibited by these drugs.

Although the potentiating mechanism cannot be completely explained, possible explanations are as follows: the contractile efficacy of PE itself completely disappears in the tissue treated with PA, because PE and PA act on the same receptors. In contrast, PE and the vasodilators act at different sites. Although the PE-induced tension is relaxaed to the baseline by the vasodilators, the contractile efficay of PE itself is not impaired by the agnets at all and still remains in the tissue. Thus, a potentiation mechansim of the UVR-induced contraction after addition of the vasodilators may be, at least in part, due to lowering of the PE-induced tension level.

ACh elicited abrupt and transient inhibition of sustained contraction induced by PE in endothelium-intact preparation. PE-induced tension was imediately relaxed to the baseline upon termination of UVR. It is well known that ACh causes release of EDRF from endothelium and relaxes endothelium-intact vascular tissues. In addition, EDRF-induced relaxation results from guanylate cyclase activation (Diamond & Chu. 1983; Rapoport & Murad, 1983; Vanhoutte *et al.*, 1986). In the present study LY83583 potentiated mark-

edly the PE-induced tension, and reversed the UVR-induced contraction to a slight relaxation in endothelium-intact rings. On the other hand, PE-induced tension in endothelium-removed tissue was slightly potentiated by LY83583. These results suggest that PE produces contraction of vascular tissue, and simultaneously rleases EDRF or activates guanylate cyclase Which will reduce the contraction of the endothelium-intact ring, and that UVR inhibits, at least in part, the release of EDRF and/or guanylate cyclase activation.

In addition to EDRF, the endothelium also releases some vasoconstrictor substances (EDCFs) which mediate contractile response of blood vessels (De Mey and Vanhoutte, 1982; Rubanyi and Vanhoutte, 1985; Hickey *et al.*, 1985). Recently EDCFs have been devided into at least three subtypes (Rubanyi, 1988; Greenberg & Diecke, 1988; Sanchez-Ferrer & Marin, 1990). Among them $EDCF_1$ is a cycloxygenase metabolite of arachidonic acid (Miller & Vanhoutte, 1985; Vanhoutte *et al.*, 1986), and $EDCF_2$ is a polypeptide that has been isolated and named endothelin (Gillespie *et al.*, 1986; Yanagisawa *et al.*, 1988), and $EDCF_3$ is an unidentified substance (Rubbanyi & Vanhoutte, 1985; Harder, 1987; Harder *et al.*, 1989). So, another possible explantation is that UVR may stimulate synthesis of some EDCFs in endothelium. While the constrictor activity of endothelin in vitro slow in onset, long-lasting, and extremely difficult to wash out (Yanagisawa & Masaki, 1989), the duration of the UVR-induced contraction in the present work is extremely short. Taken together, the UVR-induced contraction may not be mediated through endothelin ($EDCF_2$) but through $EDCF_1$ and/or $EDCF_3$.

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= 국문초록 =

흰쥐 적출 흉부대동맥근의 자외선 수축반응에 관하여

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자외선조사는 흰쥐흉부대동맥의 휴지기장력에 거의 영향을 미치지 못했으나, phenylephrine으로 수축된 표본에서는 자외선조사로 내피세포가 존재하면 수축반응을, 내피세포가 제거되면 이완반응이 나타났다. 이 수축반응은 조사시간의 길이(10~320초)에 비례하여 증가하였으나 이완반응은 그렇지 못하였다. 내피세포 존재표본에서 자외선의 수축반응은 phenylephrine농도의 증가(10^{-7}~10^{-5} M) 그리고 acetylcholine(10^{-6} M), isoproterenol(10^{-7} M) 및 nitroglycerin(3.5×10^{-8} M)의 추가투여시 크게 강화되었다. 그러나 phentolamine(10^{-6} M) 또는 LY83583(10^{-7}, 10^{-6} M)의 추가투여시에는 자외선 수축반응이 억제 또는 이완반응으로 역전되었다. 내피세포 제거표본에서의 자외선 이완반응은 phenylephrine농도의 증가 그리고 isoproterenol, nitroglycerine, phentolamine 및 LY83583의 추가투여시 유의하게 감약되었다.

이상의 성적은 흰쥐 적출 흉부대동맥에서 자외선조사는 내피세포 존재유무에 따라 수축과 이완반응이 각각 나타나며, 수축반응은 자외선에 의한 EDRF 유리억제 또는 부분적으로 어떤 EDCF와도 관련이 있음을 시사하고 있다.